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Evaluation of vaginal cytology as a monitoring tool in canine transmissible venereal tumor in bitch during vincristine sulfate therapy

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Abstract
Canine Transmissible Venereal Tumor (CTVT) is a naturally occurring contagious neoplasm that primarily affects sexually active dogs. Chemotherapy with vincristine sulfate remains the treatment of choice with high success rates. This study evaluated the use of vaginal cytology as a simple, non-invasive tool to monitor the therapeutic response of five bitches diagnosed with CTVT undergoing vincristine sulfate therapy. Vaginal smears were collected before and after each weekly dose and evaluated microscopically. A progressive decline in neoplastic cell population with a corresponding increase in superficial epithelial cells was observed, correlating with clinical tumor regression. Vaginal cytology may serve as a valuable adjunct to clinical evaluation during therapy.

Keywords: CTVT, vincristine, vaginal cytology, canine, chemotherapy monitoring, tumor regression

Introduction
Canine transmissible venereal tumor (CTVT), first described by Novinsky in 1876, is a contagious round cell tumor primarily transmitted during coitus (Das and Das, 2000; Ganguly *et al*., 2016) [2, 3]. It commonly affects the external genitalia but can also involve mucous membranes and subcutaneous tissues (Strakova and Murchison, 2015) [9]. Cytologically, CTVT is characterized by large round cells with abundant vacuolated cytoplasm and prominent nucleoli (Beach *et al*., 1983) [1].
Vincristine sulfate, a vinca alkaloid, disrupts microtubule assembly and is the most effective chemotherapeutic agent for CTVT, inducing remission in 90-100% cases (Rogers *et al*., 1998; Sudhakara Reddy *et al*., 2017) [7, 10]. Although tumor size reduction is typically used to monitor response, cytological monitoring may provide earlier, objective indicators of therapeutic efficacy (Mukaratirwa *et al*., 2005; Gharagozlou *et al*., 2008) [6, 4].
Vaginal cytology, routinely used in reproductive management, can be utilized for monitoring cytological changes in CTVT during therapy (Vail and Thamm, 2019) [11]. This study aims to evaluate vaginal cytological changes after each vincristine sulfate dose and correlate them with clinical response in five dogs.

Materials and Methods
Case Selection
Five adult female dogs, aged between 2-5 years, weighing 20-30 kg, presented to the Veterinary Clinical Complex with clinical signs of genital masses, hemorrhagic discharge, and confirmed diagnosis of CTVT via Vaginal cytology.

Diagnostic Criteria
Diagnosis was based on

- Clinical examination (protruding friable mass)
- The diagnosis of canine transmissible venereal tumor (CTVT) can be efficiently established by vaginal cytology, which reveals distinctive cytological features characteristic of the tumor. The smears commonly exhibit large, round to oval tumor cells with centrally located round nuclei, coarse chromatin, and moderate to abundant

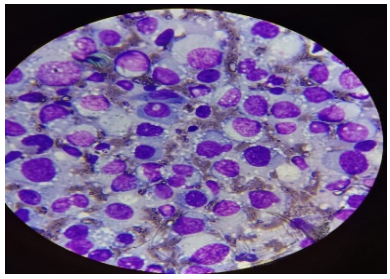
pale basophilic cytoplasm containing multiple clear cytoplasmic vacuoles (Das & Das, 2000; Moulton, 1990) [2, 5]. The cells are generally uniform with high cellularity and frequent mitotic figures, indicating active proliferation (Beach, 1983) [1]. Inflammatory cells, predominantly lymphocytes and neutrophils, may be present in the background depending on the degree of local tissue reaction (Das & Das, 2000) [2]. Vaginal cytology thus serves as a rapid, minimally invasive, and reliable diagnostic tool for confirming CTVT during clinical evaluation (Moulton, 1990) [5].

Treatment Protocol

All five dogs were administered vincristine sulfate intravenously at 0.025 mg/kg (0.6 mg/m²) body weight once weekly. Treatment continued until complete clinical remission was achieved.

Cytological Monitoring

- Vaginal cytology was performed prior to the first dose and 7 days after each subsequent dose.
- Smears were prepared using sterile cotton swabs, air-dried, methanol-fixed, and stained with Giemsa.
- At least 200 cells were counted per slide, classifying them as:
- Tumor cells (round cells with vacuolated cytoplasm)
- Parabasal, intermediate, and superficial epithelial cells
- Inflammatory cells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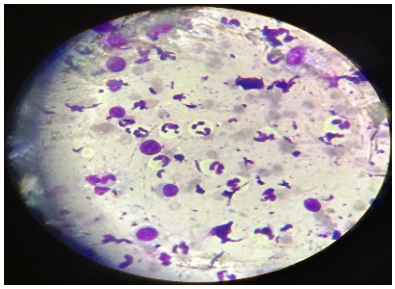
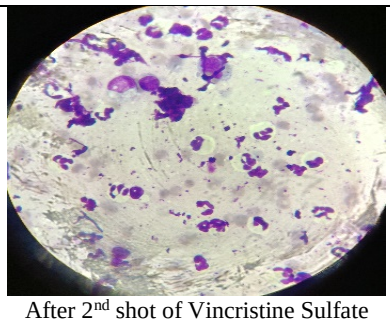

After 1st shot of Vincristine Sulphate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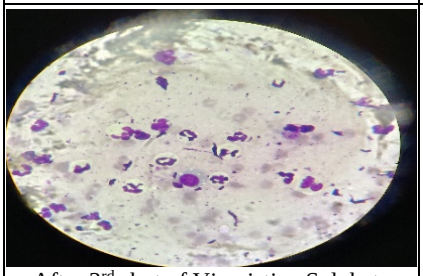
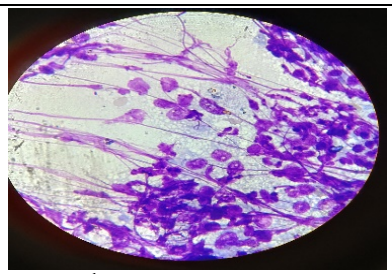

After 3rd shot of Vincristine Sulphate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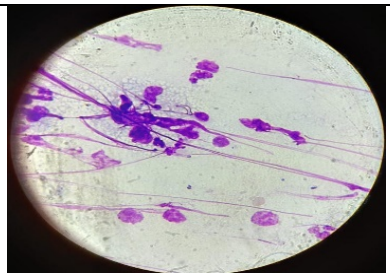
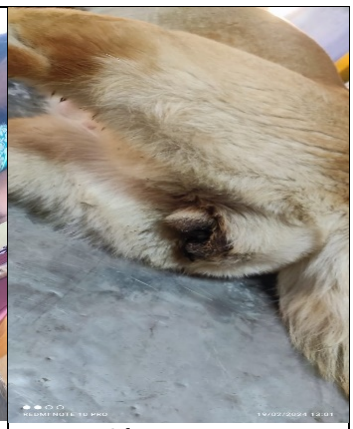

After few days

Clinical Monitoring

Tumor size was measured using calipers weekly.

Before treatment

Before treatment


After treatment

Dose	Mean Tumor Size (cm²)
Before therapy	15.0 cm²
After 1st dose	10.0 cm²
After 2nd dose	5.5 cm²
After 3rd dose	2.0 cm²
After 4th dose	0.0 cm²

Results

Dose	Mean% Tumor Cells	Mean% Superficial Cells	Inflammatory Cells
Before Therapy	85%	5%	Present
After 1st Dose	60%	20%	Mild
After 2nd Dose	35%	45%	Minimal
After 3rd Dose	10%	70%	Absent
After 4th Dose	0%	90%	Absent

- **Clinical Response**

a) Tumor size reduced significantly after each dose.
b) All five cases showed complete clinical remission after 4 doses.

- **Cytological Changes**

a) Progressive decrease in tumor cells
b) Increase in superficial epithelial cells
c) Disappearance of inflammatory cells with therapy progression

Data Analysis

Descriptive analysis of the data was carried out to evaluate the pattern of tumor regression both clinically and cytologically. In all five cases, a consistent progressive decrease in tumor size was observed after each vincristine sulfate injection.

Cytological smears showed a gradual decline in the percentage of neoplastic round cells after each dose, accompanied by a corresponding increase in the proportion of superficial vaginal epithelial cells. Initially, smears revealed approximately 85% neoplastic cells with only 5% superficial epithelial cells and presence of inflammatory cells. By the end of the third week, tumor cells were rarely observed, and smears were dominated by superficial epithelial cells (>70%) with absence of inflammation. Complete cytological remission was achieved in all animals after the fourth dose, with the absence of tumor cells and a cytology pattern resembling normal estrus smears.

The clinical regression of tumor size as measured by calipers closely paralleled the cytological changes. All animals achieved complete clinical remission by the fourth week of therapy. The consistency between the cytological findings and tumor size reduction suggests a strong positive association between cytological improvement and clinical response.

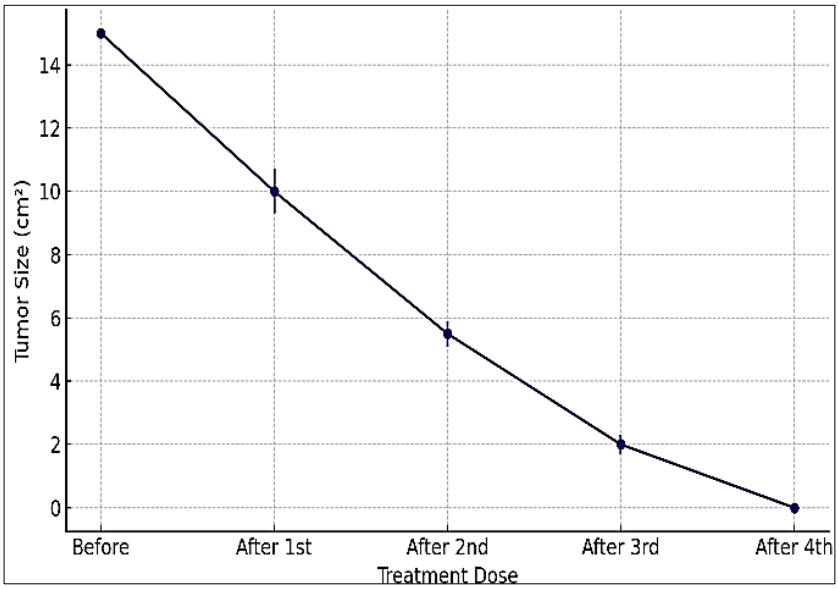


Fig 1: Tumor Size Reduction During Vincristine Therapy

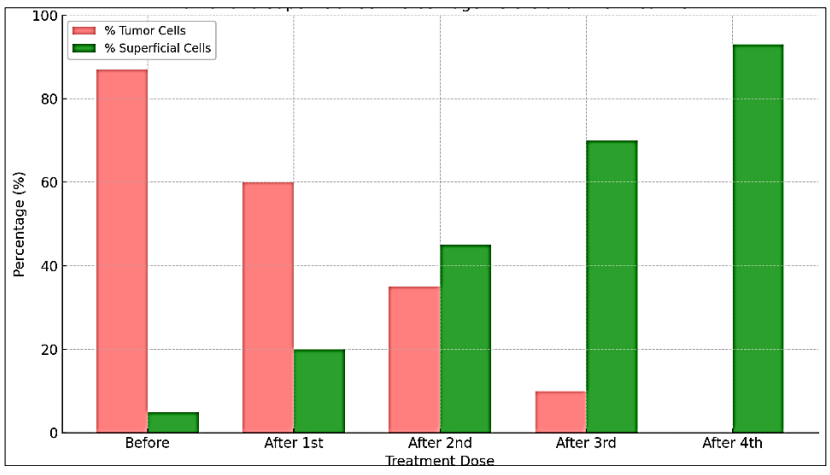


Fig 2: Cytological Changes During Vincristine Therapy

Discussion

The cytological features observed during treatment are consistent with the known regression pattern of CTVT under vincristine therapy (Rogers *et al*., 1998; Mukaratirwa *et al*., 2005) [7, 6]. Vaginal cytology offers a valuable, non-invasive adjunctive method to clinical evaluation.

Early reduction in neoplastic cells, even before complete clinical regression, provides an early indication of therapeutic efficacy, as observed by Gharagozlou *et al*. (2008) [4] and Singh *et al*. (2013) [8]. The progressive increase in superficial epithelial cells indicates restoration of normal vaginal mucosa post tumor regression (Vail and Thamm, 2019; Romagnoli and Sontas, 2010) [11, 12].

The absence of inflammatory cells after the second dose further supported ongoing tumor regression, consistent with findings of Moulton (1990) [5] and Das *et al*. (2000) [2].

Vaginal cytology, being simple, rapid, and cost-effective, can be particularly useful in field and resource-limited settings where repeated biopsies or advanced imaging are impractical.

Conclusion

Vaginal cytology is an effective, non-invasive monitoring tool to evaluate therapeutic response during vincristine sulfate chemotherapy in CTVT-affected bitches. It allows early detection of cytological remission prior to complete clinical regression and may help guide optimal treatment duration.

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